Journal of Medical Virology 50:176–180 (1996)

Detection of Human Viruses Using Primary Cells Immortalised by Oncogene Transfection, in Comparison With Primary Cells and Established Cell Lines

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No single established cell line was found capable of substituting for primary baboon kidney (PBK) or primary rhesus macacque kidney (PRK) cells for detection of human viruses. Although a panel of cell lines could detect influenza, parainfluenza, and enteroviruses, which are among the most important viruses encountered in routine diagnostic laboratories, the sensitivity of this panel was not as high as that of PBK or PRK cells. However, in a promising complementary approach, PBK and PRK cells have been immortalised successfully by oncogene transfection, and some of the resulting cell lines have retained susceptibility to human viruses, and may be suitable for routine diagnostic use.

KEY WORDS: immortalised cells, cell lines, virus detection

INTRODUCTION

Diagnostic laboratories in the United Kingdom use primary baboon kidney (PBK) and primary rhesus kidney (PRK) cells as their main substrate for virus detection. Unfortunately, these primary cells have only limited subculture capability. The continuous provision of fresh supplies raises ethical and practical considerations which impel a search for alternatives. Direct techniques, such as immunofluorescent staining and nucleic acid hybridisation, require virus-specific probes [Yolken et al., 1989] and would fail to detect an unsuspected virus or multiple infection [Subbarao et al., 1989]. Attempts were therefore made to derive a cell-based system to replace PBK and PRK cells. As no single cell line appeared to have the same sensitivity to different virus groups, a first approach was to attempt to identify a panel of cell lines, which between them would detect the viruses likely to be encountered. A second was to immortalise PBK and PRK cells by transfection with selected oncogenes [Land et al., 1983; Ruley, 1983]. This technology has enabled the successful development of cell lines which retain differentiated biochemical and other features of their parent primary cultures [Scott et al., 1986; Beito et al., 1993; Viallet et al., 1994]. Such cells may also retain susceptibility to viruses [Choi et al., 1990]. The feasibility that immortalised baboon and rhesus kidney cells could retain high virus susceptibility was investigated.

MATERIALS AND METHODS

Cells and Viruses

Established cell lines, PBK cells, and PRK cells were obtained from the Cell Resources Department, Division of Scientific Resources, Porton Down, Salisbury, UK. Viruses were obtained from the Central Public Health Laboratory, London, UK.

Media and Chemicals

Cell culture media and foetal calf serum were obtained from Life Technologies, Paisley, UK. Geneticin (G418) and calcium chloride were purchased from Sigma Chemical Co. Poole, UK.

Cell Immortalisation

The primary cells were immortalised by transfection with the plasmids pUK42, pUKEδt, and pUKEori-. Their construction has been described elsewhere [Kreuzburg-Duffy and MacDonald, 1994]. These contain the large T-antigen region of the SV40 genome, and also the gene

Accepted for publication June 4, 1996.

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The authors wish to record their shock and deep regret at the death of Ute Kreuzberg-Duffy after a road accident on 4th May 1996.

TABLE I. Sensitivity of Different Cell Lines to Influenza A Taiwan 1/86*

Cell line	No. of experiments	72 hr Mean	72 hr SD	168 hr Mean	168 hr SD
PMK	9	3.4	1.5	4.2	1.9
MDCK	11	1.4	1.5	3.7	2.4
LLC-MK2	3	<0		<0	
RPMI	2	<0		<0	
Vero C1008	2	<0		<0	
Mv-1-Lu	2	2.3	0.8	4.3	0.3
MA104	2	<0		<0	
Vero 76	4	<0		<0	
Chang Conjunctiva	4	2.0	2.4	2.3	2.3
Sp-1-K	2	<0		1.3	1.3
Mpf	2	0.3	0.3	1.0	1.0
BGM	1	<0		<0	

*Log maximum dilution at which CPE is discernible.

pSV2-neo which confers resistance to geneticin (G418). The plasmids are origin deleted to prevent extrachromosomal replication. PBK and PRK cells were cultured in M199 plus 10% newborn calf serum. Transfection was achieved by a standard calcium phosphate precipitation technique [Graham and van der Eb, 1973]. Cell colonies resistant to G418 were isolated using cloning rings, subcultured, and developed into cell lines.

Virus Susceptibility Testing

Cells were grown at 37°C in either roller tubes or microwell trays containing the appropriate medium plus 10% foetal calf serum. PBK, PRK, LLC-MK2, and Chang conjunctiva cells were cultured in M199 medium; MA104 and RPMI cells in RPMI 1640 medium; and MDCK, Vero C1008, Vero 76, Mv-1-Lu, Sp-1-K, Mpf, and BGM cells in minimal essential medium (MEM).

The cells were infected subsequently with increasing 10-fold dilutions of standardised virus preparations in serum-free media at 35°C. After 3, 7, and 10 days, the cells were examined microscopically for the presence of cytopathic effect (CPE). Haemadsorbing activity in cells infected with influenza and parainfluenza was examined microscopically after washing the cells with phosphate-buffered saline (PBS) and flooding with a 0.5% suspension of washed guinea pig erythrocytes. Haemagglutinating activity in supernatant media from the same cells was titrated in 96-well microtitre trays, using washed guinea pig erythrocytes in a standard procedure [Barrett and Inglis, 1985].

RESULTS

Susceptibility of Candidate Established Cell Lines to Selected Viruses

Cell lines were tested initially for susceptibility to an influenza A laboratory strain (Taiwan 1/86; Table I). PMK cells developed CPE earliest, but by 168 hr, MDCK and Mv-1-Lu cell lines had detected similar levels of virus. Chang conjunctiva cells were only sensitive in some experiments. BGM cells have been reported to be sensitive to enteroviruses, but not to influenza viruses [Dahling and Wright, 1986]. In these experiments they were infected with Coxsackie B3 only and were found to be highly susceptible.

MDCK and Mv-1-Lu were infected next with two influenza A clinical isolates (A47435/89NPA and A45115/89T/S), an enterovirus (Coxsackie B3), and parainfluenza 2 and 3 (Table II). MDCK cells were susceptible to both influenza clinical isolates, and also to parainfluenza 3 but not 2. Mv-1-Lu cells were susceptible to the influenza isolates only. BGM were highly susceptible to Coxsackie B3. The PBK controls were more sensitive than the established cell lines to all the test viruses.

Preliminary Investigation of Immortalised Cell Lines: Virus Susceptibility of PBK Cells Immortalised by Transfection With pUKEδt

The sensitivity of five cloned cell lines from one culture after transfection with pUKEδt is shown in Table III. All five lines were susceptible to Coxsackie B3, with a similar degree of sensitivity to PMK cells. In addition, three of the lines were susceptible to influenza A Taiwan, although in one line (δt12D17) this was only just discernible. One of the lines (δt12D19) approached previous PBK levels of sensitivity, although in the present experiment the control PMK cells were more highly sensitive than usual.

Virus Susceptibility of Immortalised PBK Cell Lines Obtained by Transfection With pUKEδt, pUKEori-, and pUK42 Thirty immortalised cell lines were established from cultures of PBK cells after transfection with pUKEδt, pUKEori-, and pUK42. To accommodate this number and to include all the available viruses, the procedure for virus susceptibility testing was shortened. The virus panel consisted of Coxsackie B3, parainfluenza 2, parainfluenza 3, and influenza A Taiwan. Cell cultures were infected only with a 1:10 dilution of stock virus, and examined for CPE after 4, 7, and 10 days. As with the established cell lines, cultures infected with parainfluenza and influenza were examined for haemadsorbtion, and the haemagglutination titres of their supernatant fluids were determined.

Results from the most susceptible immortalised ba-

TABLE II. Sensitivity of Selected Cell Lines to Two Clinical Isolates of Influenza A*

Virus	Cell line			
	PMK	MDCK	Mv-1-Lu	BGM
A4735/89NPA				
72 hr	2.0	<1.0	<1.0	ND
168 hr	3.0	2.0	2.0	
A45115/89T/S				
72 hr	3.2	<2.0	<2.0	ND
168 hr	4.2	3.2	3.2	
Coxsackie B3				
72 hr	4.0	<0	<0	3.3
168 hr	8.0	<0	<0	6.8
Parainfluenza 2				
72 hr	<0	<0	<0	ND
168 hr	7.0	<0	<0	
Parainfluenza 3				
72 hr	1.0	<0	<0	ND
168 hr	2.0	1.0	<0	

*Log maximum dilution at which CPE is discernible.

TABLE III. Sensitivity of Five Cloned Immortalised Cell Lines to Influenza A Taiwan and Coxsackie B3 Viruses*

Virus	Cell line					
	PMK	δt12D4	δt12D12	δt12D14	δt12D17	δt12D19
A Taiwan						
72 hr	3.5	<1.0	<1.0	2.0	<1.0	2.0
168 hr	8.5	2.0	<1.0	2.0	<1.0	4.0
Coxsackie B3						
72 hr	4.0	5.0	6.0	4.0	5.0	6.0
168 hr	8.5	7.0	8.0	4.0	7.0	7.0

*Log maximum dilution at which CPE is discernible.

TABLE IV. Immortalised Baboon Kidney Cells: CPE Caused by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
42/17/3	76 (21)	35 (20)	57 (21)	60 (20)
42/17/8	71 (14)	50 (12)	83 (12)	75 (12)
δt12D4	100 (9)	88 (9)	70 (10)	80 (10)
ori-11/7	75 (4)	50 (4)	75 (4)	75 (4)
PBK	100 (15)	88 (16)	88 (16)	93 (15)

*Percent cultures showing CPE (total).

TABLE V. Immortalised Baboon Kidney Cells: Haemadsorption Caused by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
42/17/3	ND	75 (20)	90 (20)	100 (20)
42/17/8	ND	75 (12)	92 (11)	92 (12)
δt12D4	ND	100 (9)	100 (10)	100 (10)
ori-11/7	ND	75 (4)	100 (4)	100 (4)
PBK	ND	92 (13)	86 (14)	92 (13)

*Percent cultures positive (total).

TABLE VI. Immortalised Baboon Kidney Cells: Maximum Haemagglutination Titres (HA units/ml) in Supernatant Fluids After Infection by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
42/17/3	ND	105 (104)/20	250 (381)/19	511 (636)/18
42/17/6	ND	85 (76)/17	218 (325)/16	373 (407)/16
42/17/8	ND	101 (158)/12	380 (680)/12	803 (846)/12
PBK	ND	1074 (1,638)/15	594 (670)/16	8576 (8,758)/12

*Mean (SD)/number of experiments.

TABLE VII. Immortalised Rhesus Kidney Cells: CPE Caused by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
Rh42/21	2/2	2/2	2/2	2/2
Rh42/22	3/3	3/3	3/3	3/3
Rh42/35	3/3	2/3	2/3	3/3
Rh42/38	2/2	1/2	2/2	3/3
Rh42/39	3/3	3/3	3/3	3/3
PRK	4/4	3/4	4/4	4/4

*Cultures showing CPE/total.

TABLE VIII. Immortalised Rhesus Kidney Cells: Haemadsorption Caused by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
Rh42/21	ND	2/2	2/2	2/2
Rh42/22	ND	3/3	3/3	3/3
Rh42/35	ND	4/4	4/4	3/3
Rh42/38	ND	2/2	2/2	2/2
Rh42/39	ND	3/3	3/3	3/3
PRK	ND	4/4	4/4	4/4

*Positive cultures/total.

boon kidney cell lines are shown in Tables IV, V, and VI. All except two lines showed complete or very highly developed CPE with Coxsackie B3, and resembled the PMK controls. With the other viruses however, the immortalised cell lines produced a much more variable CPE, and in general this was clearly less pronounced than with PMK. Four cell lines (42/17/3, 42/17/8, δ12D4, and ori-11/7) appeared to be as sensitive as PMK at exhibiting haemadsorption with the influenza and parainfluenza viruses, but the other lines showed lower sensitivities. Three cell lines (42/17/3, 42/17/6, and 42/17/8) produced markedly higher haemagglutinating titres than the remainder, although not as high as PBK. Two of these latter lines (42/17/3 and 42/17/8) had been as sensitive as PBK at exhibiting haemadsorption.

At this stage, for reasons of availability of animals, the supply of primary kidney cells was changed from baboon to rhesus macacque (PRK cells). Experience in diagnostic laboratories suggested that these may be even more sensitive to human viruses than PBK. Accordingly, about 30 immortalised cell lines were derived from PRK cell cultures, using the pUK42 construct (The pUKEδt and pUKEori- constructs in our hands were not successful in immortalising PRK cells). These PRK cell lines were screened for susceptibility to the same human viruses as the PBK cells. Results from the five most sensitive lines are shown in Tables VII, VIII, and IX. All five cell lines detected the test viruses by CPE in every trial, with the exception of Rh42/39, which failed to detect parainfluenza 2 in one of two trials. However, the PRK controls failed in one of four trials with this virus. All five cell lines detected parainfluenza 2 and 3 and influenza A by haemadsorbtion, as did the control PRK cultures. The immortalised lines gave somewhat higher supernatant haemagglutination titres with parainfluenza 2 than did PRK. With parainfluenza 3, one immortalised line was better, two were somewhat worse than the control PRK cells, and two lines were about the same. With influenza A, one line was worse, two were about the same, and two were better than the PRK cells. The variation in haemagglutination titres between experiments was high in these experiments, as shown by the large standard

TABLE IX. Immortalised Rhesus Kidney Cells: Maximum Haemagglutination Titres (HA units/ml) in Supernatant Fluids After Infection by Test Viruses*

Cell line	Virus			
	Coxsackie B3	Parainfluenza type 2	Parainfluenza type 3	Influenza A Taiwan
Rh42/21	ND	3,200 (−)/2	416,000 (−)/2	40,800 (−)/2
Rh42/22	ND	1,200 (58)/3	34,933 (28,175)/3	19,200 (64,000)/3
Rh42/35	ND	800 (730)/3	15,660 (1,208)/3	18,347 (7,715)/3
Rh42/38	ND	3,200 (−)/2	84,600 (−)/2	67,200 (−)/2
Rh42/39	ND	2,933 (3,029)/3	70,827 (116,031)/3	147,200 (22,726)/3
PRK	ND	600 (231)/4	84,600 (49,622)/4	56,000 (65,137)/4

*Mean (SD)/number of experiments.

deviations. Nevertheless, three of the five rhesus lines (Rh42/21, Rh42/35, and Rh42/39) were the same as or better than PRK in detecting the test panel of viruses in all the procedures used. Taken together, these findings suggest that the best immortalised rhesus kidney cell lines possess sensitivity to human viruses that is very similar to that shown by PRK cells.

DISCUSSION

The present results support earlier indications that no single existing established cell line can by itself substitute for PMK cells. The panel of cell lines MDCK, Mv-1-Lu, and BGM between them detected influenza, parainfluenza, and enteroviruses (among the most important viruses requiring routine diagnosis), but was less sensitive than PBK cells. An alternative approach was to immortalise susceptible primary cell cultures by transfection with plasmid constructs containing selected oncogenes. This has already proved successful with swine testicle cells, which retained sensitivity to certain porcine viruses [Choi et al., 1990]. Here, initial findings showed that some PMK cells immortalised with the oncogene construct pUKEδt retained susceptibility to influenza A Taiwan and Coxsackie B3 viruses. An extended trial of 30 baboon kidney cells lines immortalised by all three oncogene constructs revealed further cell lines which supported the test viruses, although none were as sensitive as PBK cells. However, a similar trial of 30 immortalised primary rhesus kidney cells has identified five cell lines that appear as sensitive as primary rhesus kidney cells, and these are currently being evaluated by diagnostic laboratories in practical situations. In addition, some of these cell lines could be valuable tools for comparative studies on the interaction of viruses with host cells.

ACKNOWLEDGMENTS

The authors are grateful to the Public Health Laboratory Service and the Home Office for financial support.

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